Use of Recombinant Human Interleukin-2 in Conjunction With Syngeneic Bone Marrow Transplantation in Mice as a Model for Control of Minimal Residual Disease in Malignant Hematologic Disorders

By A. Ackerstein, E. Kedar, and S. Slavin

Unlike allogeneic bone marrow transplantation (BMT), autologous BMT is not accompanied by immune-mediated graft-versus-leukemia (GVL) effects; hence, the relapse rate observed after autologous BMT in malignant hematologic disorders is higher than that observed after allogeneic BMT. Autologous BMT represents a much safer medical procedure available for many patients in need in situations where allogeneic BMT is not feasible or risky. The present experiments were designed to investigate whether it might be possible to combine the therapeutic benefits of autologous BMT with additional immunotherapy after BMT. The tumor model used for investigating GVL effects was the murine B-cell leukemia (BCL1), a spontaneous, nonimmunogenic, highly lethal leukemia of BALB/c origin. BALB/c mice inoculated with $10^6$ BCL1 leukemia cells were treated on day -1 with cyclophosphamide 100 mg/kg and transplanted with normal syngeneic BM cells on day 0. High-dose recombinant interleukin-2 (rIL-2) ($100,000$ Cetus units $\times 3$/day intraperitoneally $\times 5$ consecutive days) was initiated on day +1, +7, or +21 after BMT. Kinetics of lymphocyte reconstitution after syngeneic BMT indicated a steep increase in the absolute number of peripheral blood lymphocytes on days 17 through 24. All experimental groups were observed for relapse. Mice receiving no rIL-2 therapy relapsed and died within 50 days after BMT, whereas mice receiving rIL-2 showed long-term disease-free survival. Optimal time for administration of rIL-2 was noted at 3 weeks post-BMT, with 90% of the mice surviving with no evidence of disease for more than 1 year. Similarly, when $10^6$ BCL1 cells were given 1 day after syngeneic BMT to simulate minimal residual disease after syngeneic BMT, rIL-2 therapy administered at 14 days post-BMT seemed effective in prolonging disease-free survival in contrast to the same regimen given at 1 day after BMT. Our results suggest that in combination with rIL-2, BMT should be further investigated as a new immunotherapeutic tool for decreasing the relapse rate after BMT for hematologic malignancies.

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DISEMINATED malignant hematologic disorders and certain disseminated chemosensitive solid tumors that cannot be successfully treated by conventional doses of chemotherapy may still be responsive to myeloablative doses of chemotherapy or chemoradiotherapy followed by rescue with autologous bone marrow transplantation (BMT). Despite the use of maximal tolerable doses of chemoradiotherapy, even in the setting of autologous BMT, it is unrealistic to expect eradication of the last tumor cell; further dose escalation is limited by potential toxicity to nonhematopoietic organs, such as the lungs and the gut. Whereas a substantial proportion of patients may be induced into a long-term unmaintained complete remission after autologous BMT, many others still relapse. In view of the limitations of existing anticancer therapeutic agents, the most realistic goal in attempting to control residual clonogenic tumor cells at the state of minimal residual after autologous BMT may involve the use of anticancer immuno-therapy. As the majority of spontaneously arising tumors are nonimmunogenic, or at best very weakly immunogenic, elicitation of antigen-nonspecific, major histocompatibility complex (MHC)-nonrestrictive cell-mediated antitumor effector mechanisms may be helpful in inducing clinically significant antitumor responses. Encouraging pioneering studies of Rosenberg led to multiple clinical trials in cancer patients using recombinant human interleukin-2 (rIL-2) alone or in combination with rIL-2-activated killer cells (LAK) or other cytokines. Although tumor regression could be documented in a considerable number of patients, induction of durable complete remission is unlikely in patients with a large tumor burden. The present experiments were designed to investigate the use of rIL-2 for induction of antileukemia effects in a murine model of nonimmunogenic B-cell leukemia/lymphoma (BCL1) at the state of minimal residual disease after syngeneic BMT.

MATERIALS AND METHODS

Animals. BALB/c mice, males, aged 8 to 16 weeks, were used throughout the experiments. The mice were kept in a standard animal facility and fed at libitum in a nonprotective environment.

Murine BCL1. BCL1, a spontaneous B-cell leukemia/lymphoma, was maintained by in vivo intravenous (IV) passages of approximately $10^6$ leukemia cells obtained from the peripheral blood of mice showing extreme lymphocytosis ($>20,000/mm^3$). Leukemia was defined by monomorphic lymppholocytosis as soon as peripheral blood lymphocyte counts exceeded 20,000/mm$^3$. According to our previous data, splenomegaly can be documented before lymphocytosis. However, as soon as the peripheral blood count reaches 20,000/mm$^3$, there is a steep increase in the number of leukemia cells, with counts normally exceeding 200,000/mm$^3$. All leukemic mice developed marked splenomegaly, which was easily palpable. All mice were observed for development of leukemia on a weekly basis using venipuncture from the retro-orbital veins through a heparinized glass capillary. Mice were monitored daily for survival.

rIL-2. rIL-2 ($3.0 \times 10^6$ Cetus units/mg, >97% pure) was kindly
supplied by Dr C.R. Franks (EuroCetus, Amsterdam, The Netherlands). High-dose rIL-2 regimen consisted of 3 daily intraperitoneal (IP) injections of 100,000 Cetus units (600,000 IU) for 5 consecutive days.

Cytoreductive chemotherapy. Cyclophosphamide (Taro, Haifa, Israel) was freshly dissolved in water before injection and administered at a dose of 100 or 200 mg/kg IV.

BMT. BALB/c bone marrow cells were obtained by flushing femora and humera using a 25-gauge needle connected to a syringe with RPMI 1640 medium. Ten million cells, in 0.25 mL, were injected IV.

Statistics. The Wilcoxon rank test was used to determine the significance of differences in survival time between treatment groups. Two-sided P values are presented. No mice were excluded from the statistical evaluation.

RESULTS

Antileukemic effects after in vivo administration of rIL-2. A total dose of 10^2, 10^3, or 10^4 BCL1 cells was inoculated IV, each dose into three groups of BALB/c mice (10 mice per group). Controls received BCL1 without additional immunotherapy. Experimental groups received rIL-2 therapy consisting of three daily IP injections of 100,000 Cetus units for 5 consecutive days, starting 1 day after tumor inoculation. As shown in Table 1, all controls developed leukemia and died in ≤70 days. Treatment with rIL-2 resulted in a marked therapeutic effect with operational cure (>2 years disease-free survival) of mice receiving 10^3 and 10^4 BCL1 cells. Treatment with the same regimen of rIL-2 failed to prevent or to alter the course of leukemia in mice receiving a BCL1 challenge of 10^6 cells (P > .05).

Despite the long-term disease-free survival in the group of rIL-2-treated mice, prevention of development of leukemia was not a result of complete eradication of tumor cells. Adoptive transfer of 10^5 spleen cells, obtained 6 months after successful rIL-2 therapy from mice with no evidence of disease, into normal BALB/c recipients resulted in rapid development of leukemia in all untreated secondary mice and similarly to the development of leukemia in controls after inoculation of 10^5 fresh leukemia cells (Table 2).

Therapeutic effects of rIL-2 after syngeneic BMT. The potential therapeutic effects of rIL-2 were further tested in conjunction with syngeneic BMT as a function of the time of administration of rIL-2 after BMT, simulating an experimental model of minimal residual disease after conditioning before BMT. Forty BALB/c mice were inoculated with 10^5 BCL1 cells each. One day after inoculation, mice received IV injection of cyclophosphamide 100 mg/kg followed 24 hours later by syngeneic BMT. A group of five mice received BCL1 after autologous BMT without rIL-2 therapy. Three groups of 10 mice each received rIL-2 therapy (three daily IP injections of 100,000 Cetus units for 5 consecutive days) starting on day +1, day +7, or day +21 post-BMT. Development of leukemia and survival were observed in all experimental groups. As shown in Fig 1, all untreated controls developed overt leukemia within 50 (median 39) days after BMT. Maximal antileukemic effects were noticed in mice treated with rIL-2 3 weeks after BMT, with 90% of mice surviving greater than 17 months with no evidence of leukemia (P < .05). The antileukemia effects of rIL-2 were much less effective when treatment was initiated 1 day or at 1 week after BMT.

As can be seen in Fig 2, following profound pancytopenia in transplanted mice, a rapid increase in the number of peripheral blood mononuclear cells occurred between days 17 and 24. Thus, it appears that the time of proliferative

Table 1. Effect of rIL-2 Therapy on Development of Leukemia in BALB/c Mice Inoculated With Increasing Doses of Murine BCL1

<table>
<thead>
<tr>
<th>BCL1 Cell Dose</th>
<th>rIL-2 Therapy*</th>
<th>Onset of Leukemia</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (d)</td>
<td>Range (d)</td>
<td>Median (d)</td>
</tr>
<tr>
<td>10^2</td>
<td>-</td>
<td>47-33-67</td>
<td>57-35-70</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>No leukemia</td>
<td>&gt;2 yr (all mice)</td>
</tr>
<tr>
<td>10^4</td>
<td>-</td>
<td>28-23-31</td>
<td>31-25-53</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>No leukemia</td>
<td>&gt;2 yr (all mice)</td>
</tr>
<tr>
<td>10^6</td>
<td>-</td>
<td>17-15-25</td>
<td>18-13-29</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>16-7-27</td>
<td>17-7-30†</td>
</tr>
</tbody>
</table>

One representative experiment out of six.*Each group consisted of 10 mice. †Not significantly different from groups without any treatment or treated with IL-2 only (P > .05).

Table 2. Evidence of Dormant BCL1 Cells in Mice With Long-Term Disease-Free Survival After Successful Treatment With rIL-2

<table>
<thead>
<tr>
<th>Cells Infused to Adoptive Recipients</th>
<th>Onset of Leukemia</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (d)</td>
<td>Range (d)</td>
</tr>
<tr>
<td>10^3 Fresh BCL1</td>
<td>25</td>
<td>18-29</td>
</tr>
<tr>
<td>10^4 Spleen cells from mice inoculated with 10^6 BCL1*</td>
<td>39</td>
<td>36-49</td>
</tr>
<tr>
<td>10^5 Spleen cells from mice inoculated with 10^4 BCL1*</td>
<td>29</td>
<td>29</td>
</tr>
</tbody>
</table>

*Spleen cells were obtained 6 months after treatment with rIL-2 from mice showing no evidence of leukemia or splenomegaly (6 mice per group).
Peripheral WBC  Peripheral mononuclears

Days after BM transplantation

Fig 2. Recovery of total peripheral white blood cells (WBC) and mononuclear cells of lethally irradiated BALB/c mice after syngeneic BMT.

Lymphocytosis is also optimal for administration of rIL-2 in animals with minimal residual disease.

Relationship between the time interval after autologous BMT and the therapeutic effects of rIL-2 in a model of quantitated minimal residual disease. A model system was designed to investigate the efficacy of rIL-2 therapy at different time intervals after BMT using a known number of leukemia cells, simulating residual disease after BMT. Groups of 10 BALB/c mice each received cyclophosphamide (200 mg/kg IV) followed 24 hours later by syngeneic BMT. A fixed dose of 10⁴ BCL1 was inoculated on day +1 or +14 after BMT and one half of the mice in each group received rIL-2 treatment (three IP injections of 100,000 Cetus units for 5 consecutive days) starting 1 day after inoculation of BCL1. As shown in Fig 3, high-dose rIL-2 therapy seemed ineffective when administered immediately after BMT. Median survival of untreated controls, untreated BMT recipients, and rIL-2–treated BMT recipients was 36, 33, and 35 days after inoculation of BCL1, respectively, and all mice died of leukemia within 50 days. Significant antileukemia effects were noted in mice receiving BCL1 and rIL-2 at 2 weeks after BMT (median survival in untreated controls, untreated BMT recipients, and rIL-2–treated BMT recipients was 44, 42, and 65 days after inoculation of BCL1, respectively) (P < .05), with some mice (20%) surviving up to 6 months.

DISCUSSION

Data presented in this report and recent data published elsewhere suggest that high-dose rIL-2 may provide antitumor effects in a murine model of leukemia/lymphoma, even against a totally nonimmunogenic tumor such as BCL1. Operational cure may be accomplished despite the continuous presence of potentially clonogenic tumor cells in the spleen of treated mice, as shown by adoptive transfer into secondary syngeneic naive recipients (Table 2). As indicated in our previous reports, although the low incidence of dormant BCL1 cells could not be detected by the fluorescence-activated cell sorter using an anti-idiotypic antibody, the presence of tumor cells could be documented by adoptive transfer experiments in vivo. Our present data suggest that high-dose rIL-2 therapy may be particularly useful in arresting minimal residual disease, a state most likely to be accomplished after high-dose chemotherapy alone or in conjunction with autologous BMT. In view of the fact that in clinical situations, too, higher-than-currently-used cytoreductive regimens are likely to produce life-threatening multisystemic complications, availability of a manipulative approach using biologic agents such as rIL-2 is likely to add another therapeutic dimension to the already-existing tumor cytoreductive regimen. In view of these facts, the use of recombinant cytokines should be further investigated subsequent to remission induction by conventional chemotherapy and particularly after autologous and allogeneic BMT, because maximal therapeutic effects of immunotherapy should be expected in the state of minimal residual disease. It is also reasonable to assume that cytokine-mediated immunotherapy is likely to be more effective in patients with reconstituting immune systems not negatively affected by concomitant administration of immunosuppressive agents such as cyclosporine A and corticosteroids used for prevention of graft-versus-host disease (GVHD) after allogeneic BMT.

As indicated by data presented in Fig 1, timing of bioimmunotherapy is critical; whereas remarkable antitumor effect was accomplished when rIL-2 was administered 3 weeks after BMT, earlier administration of rIL-2 after BMT was much less efficient. As indicated by the data presented in Fig 2 and as previously published, the third week after BMT represents the time of maximal expansion of circulating lymphocytes in mice cytoreduced with cyclophosphamide or ionizing irradiation. Depression of natural
killer activity after high-dose cyclophosphamide was previously documented by Ricardi et al and Ballas, but was fully restored by day 12. These observations may explain the effectiveness of later administration of rIL-2. From a clinical point of view, administration of cytokines several weeks after BMT may be even more desirable, because at that time treated hosts may already show a substantial hematopoietic reconstitution and a more stable clinical condition.

The potential beneficial effects of rIL-2 in the setting of minimal residual disease have yet to be documented clinically, but results presented in the present work suggest that such an approach may lead to development of a state of tumor dormancy and help keep in check residual tumor cells escaping chemotherapy or chemoradiotherapy, thus reducing the incidence of relapse after conventional or high-dose cytoreductive anticancer therapy in malignant hematologic disorders and perhaps certain responsive solid tumors. After autologous BMT, potent antitumor effects may thus be accomplished with no clinically overt GVHD. Interestingly, recent data from the International Bone Marrow Transplant Registry suggests that recipients of T-cell–depleted transplants may have an increased risk of relapse even after adjustment for GVHD, suggesting additional antileukemia effects independent of GVHD.

ACKNOWLEDGMENT

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REFERENCES

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